REMARKS

The Invention

In general, the invention as presently claimed features a mouse having two transgenes. The first transgene, which includes a regulatory gene encoding a regulatory protein, is integrated into an endogenous gene such that two events occur. First, the endogenous gene is mutated by the insertion of the transgene. Second, expression of the regulatory gene is regulated by the promoter of the endogenous gene.

The second transgene includes a gene operably linked to a regulatory sequence that is under the control of the regulatory protein encoded by the first transgene; this gene is referred to hereinafter as "the regulated gene." Gene regulation may be positive (i.e., the expression of the regulated gene may be increased in the presence of the regulatory protein) or negative (i.e., the expression of the regulated gene may be decreased in the presence of the regulatory protein). In either case, because the expression pattern of the regulatory protein is determined by expression of the endogenous gene, the end result is that it is the endogenous gene's expression that determines how the regulated gene is expressed.

The Office Action

The sole rejection is of all pending claims (claims 1, 2, 6-13, 18, and 20-24) for failing to comply with the enablement rejection. Applicant respectfully traverses this rejection.

The enablement of mice having no phenotype

As an initial matter, applicant wishes to address the issue of whether the specification enables the use of a mouse having no phenotype. It is clear to applicant, upon reading the present Office action, that when referring to a mouse

having no phenotype, applicant and the Office are using the word "phenotype" differently. Applicant has used this term to mean the observable physical characteristics of an organism, such as appearance or behavior. In this context, it has been and remains applicant's position that the claimed mouse may look and behave normally and yet still be useful for all of the reasons set forth in applicant's previous replies. When the Office refers to a mouse's phenotype, the Office is considering the expression of the first and second transgenes as part of the phenotype. On this basis, the Office is rejecting applicant's contention that a mouse having no phenotype would nonetheless be useful.

In view of applicant's new-found understanding of the concerns set forth by the Office, applicant has amended the claims to clarify that only certain mice are encompassed by the claims. These mice (i) are expressing the first transgene at a level sufficient to modulate expression of the second transgene; and (ii) include a second transgene positioned such that it is capable of being expressed in a manner controlled by the first transgene. Thus, as amended, the claims do not read on mice having no phenotype, using the Office's definition of phenotype as it relates to modifications in the level of gene expression.

Predictability

In view of the present claim amendments, the only remaining issue is that of predictability, i.e., whether the claimed mice can be made and used without undue experimentation. It is not disputed that the techniques for making transgenic mice were well known as of the priority date of the present application. The Office contends, however, that the production of mice in which the first and second transgenes are expressed at "sufficient levels" is not enabled by the specification because of the influence of positional effects and the strength of the promoter of the endogenous gene. Applicant respectfully disagrees.

For the first transgene to be expressed at a sufficient level, the expressed regulatory protein should be capable of modulating expression of the second transgene. For the second transgene to be expressed at a sufficient level, the protein encoded by the transgene should be capable of playing its desired role in the cell. Applicant acknowledges that not every transgene will integrate such that the transgene is capable of being expressed at a sufficient level, but this is not the legal test. Rather, the test is whether one skilled in the art could make and use the claimed invention without undue experimentation.

In the present case, the required experimentation is routine and well known, and is not undue. Indeed, as discussed in the attached declaration from Dr. George Gaitanaris, the inventor in the present application, the prior art is replete with scientific publications describing the production of mice expressing either a transgene positioned for expression under the control of the promoter of the endogenous gene (corresponding to the "first transgene" in the claims) or a gene operably linked to a regulatory sequence that itself is regulated by a regulatory protein encoded by another transgene (corresponding to the "second transgene"). For example, in 1991, Friedrich and Soriano described the production of more than two dozen different mouse lines, each containing a reporter transgene expressed under the control of a promoter of an endogenous gene (Genes Dev. 5:1513-1523, 1991; Exhibit A). In 75% of these mouse lines, the protein encoded by the transgene could be detected (page 1517, left col.), and many of the lines were demonstrated to be mutagenic. In another study, Wurst et al. (Genetics 139:889-899, 1995; Exhibit B) produced more than 300 mouse embryonic stem (ES) cell clones containing integrations of a *lacZ* transgene. When these clones were used to make 8.5-day chimeric mouse embryos, "approximately one third of the clones showed widespread *lacZ* expression" and "[t]hirty-five...exhibited tissue specific or spatially-restricted expression patterns..." (page 895, right col.). Thus, while not describing applicant's claimed invention, these references

demonstrate that transgenes can be, and have been, successfully expressed from endogenous promoters without apparent difficulty.

Similarly, for the second transgene, Furth et al. (Proc. Natl. Acad Sci. USA 91:9302-9306, 1994; Exhibit C) produced mice carrying a reporter gene fused to seven *tet* operator sequences as well as a transgene encoding a tetracycline-controlled transactivator fusion protein (tTA) composed of the *tet* repressor and the VP16 activation domain under the control of the hCMV IE1 promoter/enhancer (page 9302, right col.). In these mice, the reporter gene was expressed in the absence of tetracycline, and repressed upon the addition of tetracycline (Tables 1 and 2). Again, of course, Furth's tTA system was not configured in the same two-transgene construct claimed by applicant. Nonetheless, this reference demonstrates that tetracycline-controlled gene expression can be, and has been, carried out in mice, again without apparent difficulty.

Moreover, while the potential problem, in some mice, of positional effects in inducible gene expression systems had been recognized, possible solutions had also been recognized. For example, Shockett and Schatz (Proc. Natl. Acad. Sci. 93: 5173-5176, 1996; Exhibit H) suggest that "[i]ntegration site-specific effects...might be overcome by surrounding individual transcription units with matrix attachment regions, shown previously to insulate stably integrated vectors and transgenes from effects mediated by cis regulatory elements adjacent to their sites of integration [citations omitted]." Insulators have since been shown to reduce positional effects in inducible gene expression systems (Exhibits I and J). Regardless, the examples provided above demonstrate that the positional effects do not hinder one's ability to successfully make the transgenic mice. Rather, positional effects become relevant when the complete lack of basal activity is required for a special purpose.

While it is true that applicant's claimed invention goes well beyond, and represents a patentable advance over, the activity described above, it is clear from the exemplary references described herein, that the production of applicant's mice could be readily accomplished using techniques known as of the priority date of the present application. Further, while the transgenes in the claimed mice are not identical to those described in the exemplary references discussed above, it is also clear from these examples that neither positional effects nor promoter strength, the two concerns raised by the Office, represent insurmountable obstacles in the production of transgenic mice.

As mice having both transgenes are produced simply by crossing a mouse having the first transgene with a mouse having the second transgene, the production of those mice cannot be questioned. The only remaining issue therefore is whether both of these transgenes can be expressed at sufficient levels in the same mouse. As is discussed in detail in the declaration from Dr. Gaitanaris, applicant's own experience in producing the claimed mice supports applicant's position that mice having sufficient first and second transgene expression can be produced without undue experimentation. In this declaration, Dr. Gaitanaris discusses the production of mouse lines having the claimed first and second transgenes, in which both endogenous alleles, in this case ApoE alleles, were inactivated by retroviral insertion and an ApoE transgene was regulated by a regulatory protein expressed from the endogenous ApoE promoter. In these mice, ApoE protein can only be produced from the transgene and this production is under the control of doxycycline, a tetracycline analog. Absence of ApoE protein is expected to lead to hypercholesterolemia. As indicated in the Gaitanaris declaration, the first transgene was expressed at a sufficient level, as demonstrated by the ability of the encoded regulatory protein to modulate expression of the second transgene. And the second transgene was expressed at a sufficient level, as demonstrated by the different cholesterol levels in the transgenic mice in the

presence and absence of doxycycline. Therefore, these results demonstrate that the two transgenes can be successfully expressed in a single mouse at sufficient levels to produce the desired phenotype. These findings support applicant's contention that the claimed mice can be made without undue experimentation and address the Office's concerns regarding predictability.

In view of the foregoing amendments and remarks, as well as the declaration from Dr. Gaitanaris, applicant respectfully requests reconsideration and withdrawal of the rejection of the claims for lack of enablement.

Conclusion

Applicant submits that the claims are now in condition for allowance, and such action is respectfully requested. If the Office deems that there are remaining issues, applicant respectfully requests a telephonic interview between the Office and the undersigned.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 6/14/05

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